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RESEARCH PAPER

Molecular identification of fungi and the types of toxins produced from contaminated corn grain in Satui, Tanah Bumbu, South Kalimantan, Indonesia

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ABSTRACT

Fungal contamination in stored corn grain not only reduces grain quality but alsoposes risks to animal and human health due to mycotoxin production. This study highlights the importance of early detection and identification of fungal pathogens in corn as a key aspect of plant protection and postharvest management, as well as the need to determine the types and concentrations of toxins produced. Corn samples were collected from a storage warehouse in Satui Village, Kota Baru Regency, South Kalimantan. Fungal isolation was conducted at the Phytopathology Laboratory, Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Lambung Mangkurat. PCR analysis and gene sequencing were performed at the Genetics Sciences Laboratory, Jakarta, while toxin type and content analyses were carried out at the Animal Husbandry Laboratory, Universitas Gadjah Mada, Yogyakarta. From 11 microbial isolates obtained from corn grain, only one fungal species was identified, namely *Aspergillus flavus*. This species was found to produce 8.00 ppb of aflatoxin, which remains below the established safety thresholds of 15 ppb for B1 and 20 ppb for total aflatoxins.

Key words: aflatoxin, *Aspergillus flavus*, corn grain, early detection, postharvest

INTRODUCTION

Corn (*Zea mays* L.) is a major staple crop that plays an essential role in global food security and serves as a primary source of livestock feed. However, its cultivation is highly vulnerable to fungal infections, which can cause significant yield losses both in the field and during post-harvest storage. Among the most important pathogens is *Aspergillus flavus*, the causal agent of corn ear rot. Previous studies, have shown that *A. flavus* infection not only reduces grain quality but also leads to the accumulation of aflatoxins—secondary metabolites that threaten plant health, animal feed safety, and human food security (Zakaria, 2024).

In addition to *A. flavus*, other fungi such as *Fusarium verticillioides* and *F. graminearum* are also major pathogens of corn, producing mycotoxins including fumonisins (FB) and deoxynivalenol (DON), which further compromise grain safety and yield (Giorni et al., 2019).

The humid tropical climate of Indonesia provides favorable conditions for fungal proliferation, particularly during drought stress, at pre-harvest stages, and under poor post-harvest management (Dharmaputra et al., 2014). Fungal pathogens in corn are generally classified as field fungi—infecting plants during crop growth—or storage fungi—developing after harvest under improper storage conditions (Sauer, 1992). *Aspergillus flavus* is categorized as a field fungus, thriving under hot and dry conditions, and frequently enters kernels through wounds caused by insect pests, mechanical damage, or environmental stress (Dharmaputra et al., 2021).

Corn ear rot caused by *A. flavus* produces visible symptoms such as yellow-green mold on kernels, premature grain drying, and reduced kernel weight, all of which diminish the quality and marketability of corn grain. Colonization may continue in storage facilities, particularly under high humidity, further increasing contamination risks (Fitriana et al., 2019). The impact is especially severe in regions with inadequate post-harvest handling, where insufficient drying and high temperatures accelerate fungal growth and mycotoxin accumulation (Galvan et al., 2010).

Effective management of *A. flavus* infection requires an integrated approach. Cultural practices such as crop rotation, field sanitation, and proper tillage can reduce soil inoculum. The use of resistant corn varieties with tighter husks and improved drought tolerance also

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lowers infection risk. Integrated Pest Management (IPM) practices, including insect pest control and the application of biological control agents, have proven effective in reducing fungal entry points and subsequent colonization (Woloshuk & Wise, 2011). Biocontrol using non-toxigenic *A. flavus* strains such as CT 3 and K49 has successfully lowered aflatoxin levels in corn by up to 86% and 60%, respectively (Abbas et al., 2006).

Therefore, this study was conducted to identify fungal pathogens associated with corn ear rot, evaluate their pathogenicity, and analyze environmental factors influencing infection dynamics in corn fields. The findings are expected to support the development of sustainable management strategies for fungal diseases in tropical corn production systems.

MATERIALS AND METHODS

Research Site and Sampling. This study was conducted in corn cultivation areas and storage facilities in the Satui region, Tanah Bumbu Regency, South Kalimantan Province. Corn ears showing symptoms of ear rot were collected from the field before harvest, while additional samples were obtained from storage facilities to assess the persistence of fungal contamination. Sampling was carried out at five distinct points, with 1 kg of material obtained from each point. Samples were placed in pre-labeled sterile bags and transported to the Phytopathology Laboratory, Department of Plant Protection, Universitas Lambung Mangkurat, for fungal isolation and identification.

Fungi Isolation and Identification. Symptomatic corn kernels with visible mold growth and decay were selected for isolation. Kernels were surface-sterilized with 1% sodium hypochlorite (NaOCl) for 1 min, rinsed three times with sterile distilled water, and placed on Potato Dextrose Agar (PDA) medium in Petri dishes. Plates were incubated at 30 ºC for 7 days, with daily monitoring of fungal growth. Emerging colonies were subcultured on fresh PDA to obtain pure isolates.

Morphological identification was conducted based on colony color, texture, and growth patterns on PDA, while microscopic characteristics (hyphal structure, conidiophore arrangement, and spore morphology) were observed using a compound microscope following the keys of Nyongesa et al. (2015).

Molecular Identification. Genomic DNA was extracted using the Quick-DNA Fungi/Bacterial Miniprep Kit (Zymo Research, California, USA) according to the manufacturer's protocol. PCR amplification targeted the Internal Transcribed Spacer (ITS) region using primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′). Each 10 µL PCR reaction contained 5 µL MyTaq™ HS RedMix (Bioline, USA), 3 µL sterile distilled water, 1 µL DNA template, and 0.5 µL of each primer.

PCR was performed in a Bio-Rad T100 thermal cycler (USA) with the following program: pre-denaturation at 95 ºC for 5 min, 30 cycles of denaturation at 95 ºC for 1 min, annealing at 54 ºC for 1 min, and extension at 72 ºC for 1 min, final elongation at 72 ºC for 5 min, and hold at 4 ºC. PCR products were analyzed by 1% agarose gel electrophoresis in 1× TBE buffer at 100 V for 30 min and visualized under UV light with a DigiDoc UV transilluminator (Biobase, China).

For species-level identification, PCR products (35 µL) were purified and sequenced at 1st Base Malaysia through PT. Genetika Science Indonesia. Sequences were analyzed using BLAST against the NCBI GenBank database. Phylogenetic analysis was performed using MEGA XI software (Tamura et al., 2021), and multiple sequence alignment was conducted using Clustal Omega and Espript 3.0 (Sievers et al., 2011; Robert & Gouet, 2014).

Pathogenicity Tests. Pathogenicity tests were conducted only for *Aspergillus flavus,* as this species is relevant to plant infection, while *Lechteimia ramosa* was excluded due to its association with human pathogenicity. Tests were carried out under laboratory and field conditions.

On Corn Seeds (Laboratory Test). Healthy corn seeds were surface-sterilized in 70% ethanol for 1 min, rinsed three times with sterile distilled water, and air-dried on sterile tissue in a laminar airflow cabinet. Sterile seeds were placed on PDA in Petri dishes (10 seeds per plate, two replicates). A control set of 10 untreated healthy seeds was maintained on sterile filter paper.

To test seed resistance to infection, three Petri dishes containing 10 seeds each were directly placed on actively growing *A. flavus* colonies (Jallow, 2021). Seeds exhibiting natural infection symptoms were also tested (10 seeds per plate, two replicates). All plates were incubated at room temperature until visible fungal growth appeared. Infection incidence and seed viability were then evaluated.

On Corn Plants (Field Test). Pathogenicity on standing plants was tested using a modified artificial

inoculation method (Marsh & Payne, 1984). Healthy ears at the silking stage (R1 growth stage) were inoculated with a spore suspension of *A. flavus* (10^6 spores/mL). Inoculated cobs were covered with sterile plastic bags for 48 h to maintain humidity and facilitate infection (Figure 1). Disease symptoms, including grain discoloration, fungal growth, and kernel rot, were assessed 10–14 days after inoculation and compared with uninoculated controls.

Aflatoxin Detections in Corn Kernels. Aflatoxin analysis was performed on infected kernels collected from both field and storage samples using Enzyme-Linked Immunosorbent Assay (ELISA) at the Animal Science Laboratory, Gadjah Mada University. The procedure followed Lee et al. (2004) with modifications. Infected samples were centrifuged, and the supernatant was subjected to ELISA using standard solutions ranging from 0 to 40 ppb. Methanol and control samples were included as comparators. Aflatoxin concentrations were determined with an ELISA reader and spectrophotometer.

RESULTS AND DISCUSSION

Morphological Identification of Fungi on Corn Grain. A total of 11 fungal isolates were obtained from corn grain samples collected from a livestock feed storage warehouse in Satui Village, South Kalimantan. Morphological identification indicated that the isolates belonged to the genus *Aspergillus* (Table 1; Figure 2). Among them, isolate JG2 was selected for further study because it exhibited colony characteristics typical of *Aspergillus*. This isolate was subjected to molecular identification and toxin analysis.

Macroscopic and Microscopic Observations. Fungal mycelium of isolate JG2 appeared on the second day of incubation, likely triggered by spore germination through enzymatic degradation of the corn kernel surface. *Aspergillus* spp. are known to produce enzymes that degrade plant cell walls, facilitating cellulose breakdown (de Vries & Visser, 2001). Within 48 hours, fungal hyphae penetrated the cotyledon tissue, and by 72 hours, colonization had spread extensively (Achar et al., 2009). After one week, colonies developed yellowish-green mycelium.

Microscopic analysis revealed branched, septate hyphae with conidiophores bearing chains of round conidia attached to vesicles (Figure 3). These morphological characteristics were consistent with previous descriptions of *Aspergillus* spp. (Natassya et al., 2013; Mizana et al., 2016; Fatmawati et al., 2018). Colony colors varied from black, green, yellow, to white depending on conidial density (Praja & Aditya, 2017). Vesicles appeared round and carried phialides (Hayani

Figure 1. Inoculated cobs covered with sterile plastic bags.

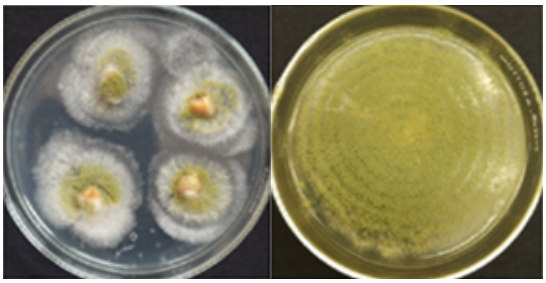


Figure 2. Macroscopic resulted of isolated fungi contaminating corn kernels (*Aspergillus* sp.).

Table 1. Results of morphological identification of fungi isolated from corn seeds

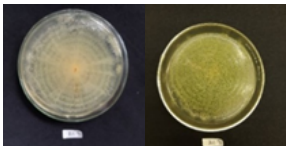
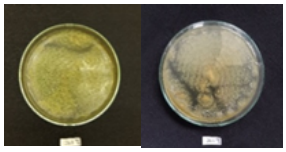
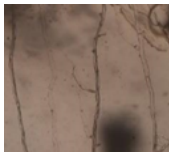
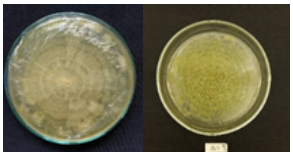
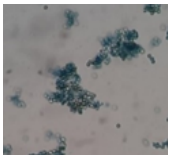
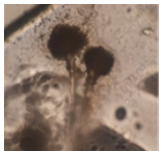
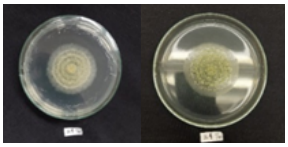
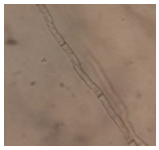
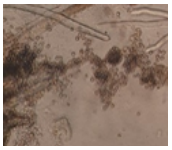
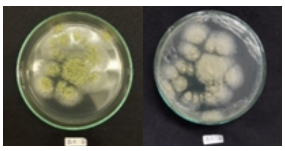
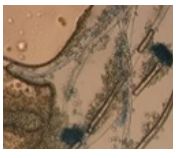
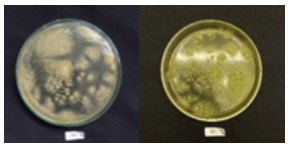
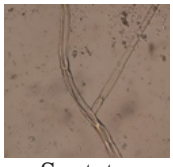
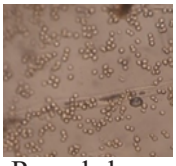
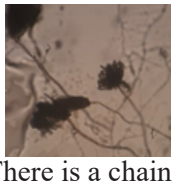
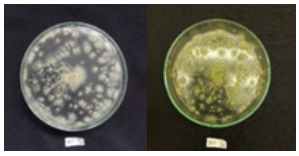
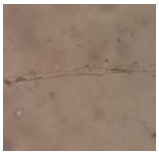
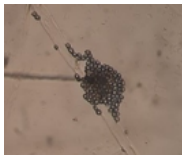
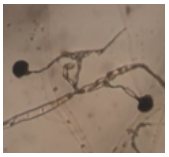
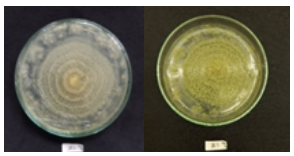
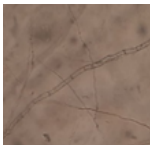
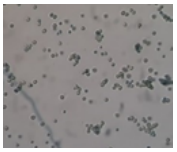
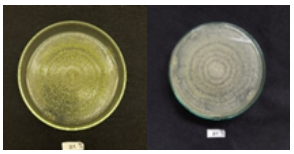
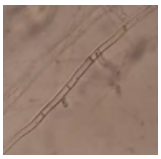
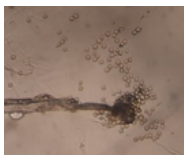
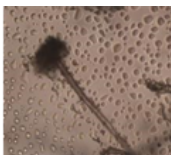
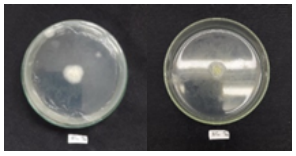
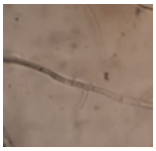
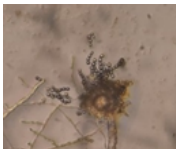
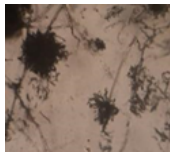
Isolate code	Macroscopic	Microscopic			Genus
		Hyphae	Spores	Conidiophores	
JG 1	 Mycelium is yellowish green	 Septate	 Round shape	There is a chain of conidia attached to the vesicle	*Aspergillus* sp.
JG 2	 Mycelium is yellowish green	 Septate	 Round shape	 There is a chain of conidia attached to the vesicle	*Aspergillus* sp.
JG 3	 Mycelium is yellowish green	 Septate	 Round shape	 There is a chain of conidia attached to the vesicle	*Aspergillus* sp.
JG 4	 Mycelium is yellowish green	 Septate	 Round shape	 There is a chain of conidia attached to the vesicle	*Aspergillus* sp.
JG 5	 Mycelium is yellowish green	 Septate	 Round shape	 There is a chain of conidia attached to the vesicle	*Aspergillus* sp.
JR 1	 Mycelium is yellowish green	 Septate	 Round shape	 There is a chain of conidia attached to the vesicle	*Aspergillus* sp.

Table 1. Continued. Results of morphological identification of fungi on corn seeds

Isolate code	Macroscopic	Microscopic			Genus
		Hyphae	Spores	Conidiophores	
JR 2					*Aspergillus* sp.
	Mycelium is yellowish green	Septate	Round shape	There is a chain of conidia attached to the vesicle	
JR 3					*Aspergillus* sp.
	Mycelium is yellowish green	Septate	Round shape	There is a chain of conidia attached to the vesicle	
JR 4					*Aspergillus* sp.
	Mycelium is yellowish green	Septate	Round shape	There is a chain of conidia attached to the vesicle	
JR 5a					*Aspergillus* sp.
	Mycelium is yellowish green	Septate	Round shape	There is a chain of conidia attached to the vesicle	

et al., 2017), key features for species-level identification.

Given the ability of *Aspergillus* spp. to produce mycotoxins in corn-based feed, molecular identification was performed to confirm species identity and assess its toxigenic potential.

Molecular Identification of Corn Grain Isolate. ***PCR Analysis Results*****.** PCR amplification of the JG2 isolate produced a distinct band at ~793 bp (Figure 4). A total of 11 fungal isolates were obtained from different points within a corn grain storage warehouse for animal feed. Based on macroscopic and microscopic characteristics, representative isolates were selected for molecular identification using ITS1 and ITS4 primers.

DNA Sequencing and Phylogenetic Analysis**.** BLAST analysis of the ITS region from isolate JG2 showed high similarity to sequences of *Aspergillus flavus* available

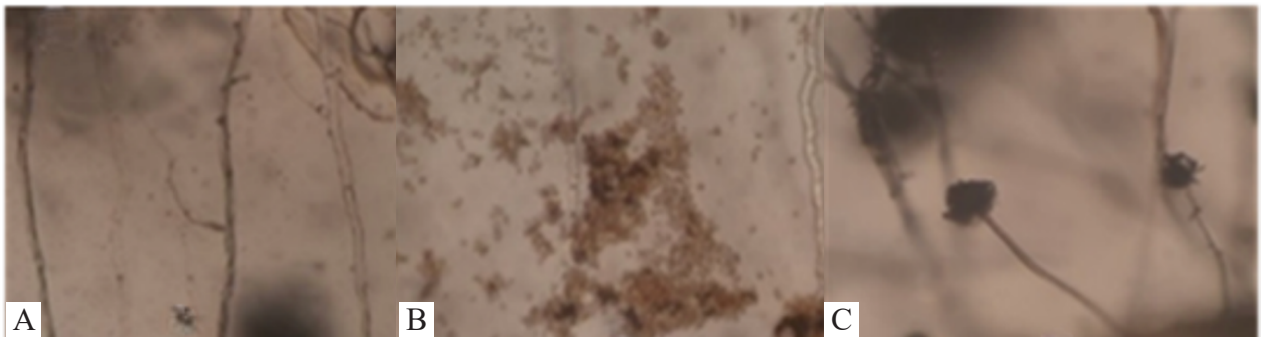


Figure 3. Microscopic characteristics of *Aspergillus* sp. isolated from contaminated corn kernels. A. Septate hyphae; B. Round vesicle; C. Chains of conidia attached to the vesicle.

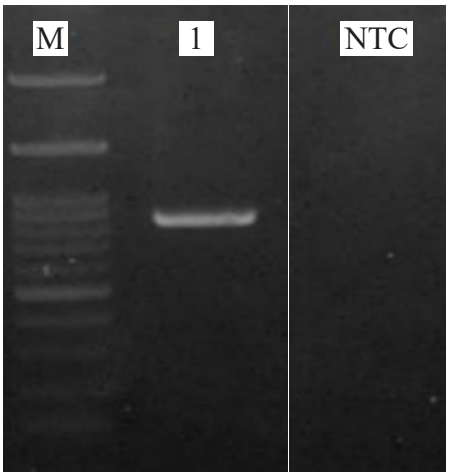


Figure 4. Gel electrophoresis of PCR products with primer ITS1/4 using (2×) MyTaq HS Red Mix. M= Marker 100 bp DNA ladder (2.5 µL); Lanes 1= JG2; NTC: Non-template control.

in the NCBI GenBank database. Phylogenetic analysis confirmed that JG2 clustered within the *Aspergillus* section Flavi, showing the closest relationship to *A. flavus* and *A. tamarii*, with the highest similarity to *A. flavus* (Figure 5). However, due to the close genetic relatedness within this section, definitive species-level identification requires additional markers such as calmodulin, β-tubulin, or actin genes.

Homology Percentage and DNA Sequence Comparison. To confirm the identification of isolates JG2, a homology percentage analysis was conducted (Table 2). Pairwise sequence comparison using the p-distance method in MEGA 11 (Tamura et al., 2021) confirmed that isolate JG2 shared 100% similarity with *A. flavus* strain Bp5 18S Qatar (KF221065.1). BLASTN hits corresponding to complete genome sequences (e.g., CP031831.1) were excluded since they differ from the partial ITS sequences analyzed. Multiple sequence alignment further verified that JG2 contained conserved regions identical to reference sequences in GenBank (Table 3; Figure 6).

Pathogenicity Test.

Laboratory Assay on Corn Seeds. Symptoms of *A. flavus* were first observed 10 days after inoculation. Infected corn seeds placed on PDA media or sterile, moistened filter paper failed to germinate and were eventually overgrown by fungal mycelium (Figure 7).

For healthy seeds inoculated with *A. flavus* on PDA, symptoms appeared within 3–7 days after inoculation. Fungal growth was visible during germination, leading to seed rot, damping-off, and in severe cases, seedling death. Infection reduced germination rates, weakened seedling vigor, and caused abnormal development, highlighting the potential for long-term yield losses when infected seeds are sown.

Infected seeds displayed characteristic discoloration, beginning with a yellowish-green surface and progressing to cotton-like growth that turned from whitish-brown to blackish. The development of dense olive-green conidial masses was consistent with cob rot symptoms caused by *Aspergillus* species (Zakaria, 2024).

Although 91.9% of corn kernels can be infected before harvest, only 3.8% show visible symptoms in the field. Laboratory assays confirmed that apparently healthy seeds are also highly susceptible to *A. flavus* infection. The risk of infection in storage increases with

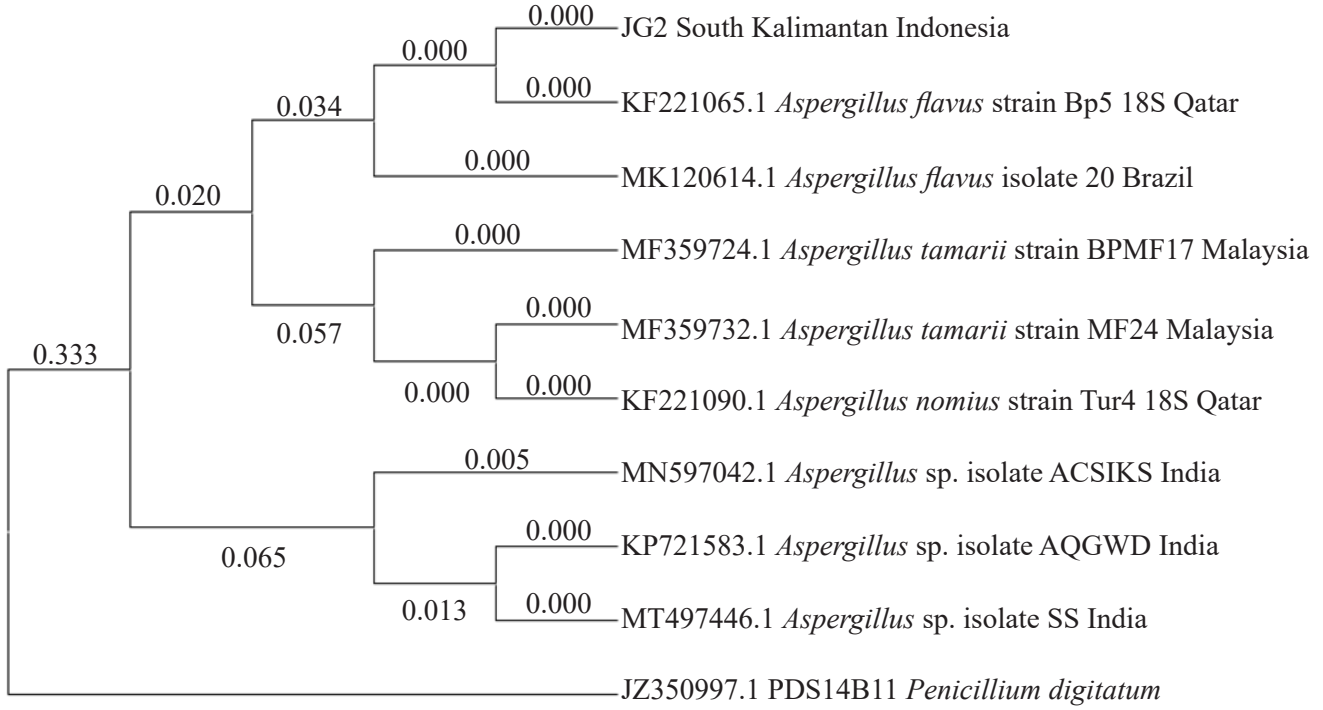


Figure 5. Phylogenetic tree showing the relationship of *Aspergillus* associated with corn kernels in Tanah Laut, Indonesia, constructed using the neighbor-joining method with 1000× bootstrap replication.

Table 2. Homology level of the JG2 sequence from South Kalimantan compared with other sequences obtained from GenBank NCBI

No	Sample origin	Accession number	Homology (%)						
			1	2	3	4	5	6	7
1.	JG2 South Kalimantan Indonesia		ID						
2.	*Aspergillus flavus* strain Bp5 18S Qatar	KF221065.1	100	ID					
3.	*Aspergillus tamarii* strain BPMF17 Malaysia	MF359724.1	98	98	98	ID			
4.	*Aspergillus tamarii* strain MF24 Malaysia	MF359732.1	98	98	98	100	ID		
5.	*Aspergillus* sp. isolate AQGWD India	KP721583.1	97	97	97	95	95	ID	
6.	*Aspergillus* sp. isolate ACSIKS India	MN597042.1	97	97	97	95	95	100	ID
7.	*Aspergillus* sp. isolate SS India	MT497446.1	97	97	97	95	95	100	100
8.	*Aspergillus flavus* isolate 20 Brazil	MK120614.1	100	100	100	98	98	97	97
9.	*Aspergillus nomius* strain Tur4 18S Qatar	KF221090.1	98	98	98	100	100	95	96

Table 3. Isolates found in feed corn kernels with pathogen identities based on ITS DNA barcodes

Isolate code	Host	Plant parts	Originated	Identity	Percentage identity to reference in NCBI (%)	GenBank Acc No.
JG2	Corn	Seed	Satui, South Kalimantan	*Aspergillus flavus*	98.94	KF221065.1

higher seed moisture content and relative humidity, which facilitate fungal growth and disease spread.

Aflatoxin production by *A. flavus* further compounds the problem, with contamination levels strongly influenced by cultivation practices, planting season, and corn variety (Williams et al., 2020). Late planting, particularly with short-duration hybrids, has been linked to a 77% increase in aflatoxin accumulation in naturally infected corn. This underscores the critical importance of both pre- and post-harvest management to reduce infection and toxin levels.

Field Assay on Corn Plants. The pathogenicity test of *A. flavus* on corn plants showed distinct outcomes in the

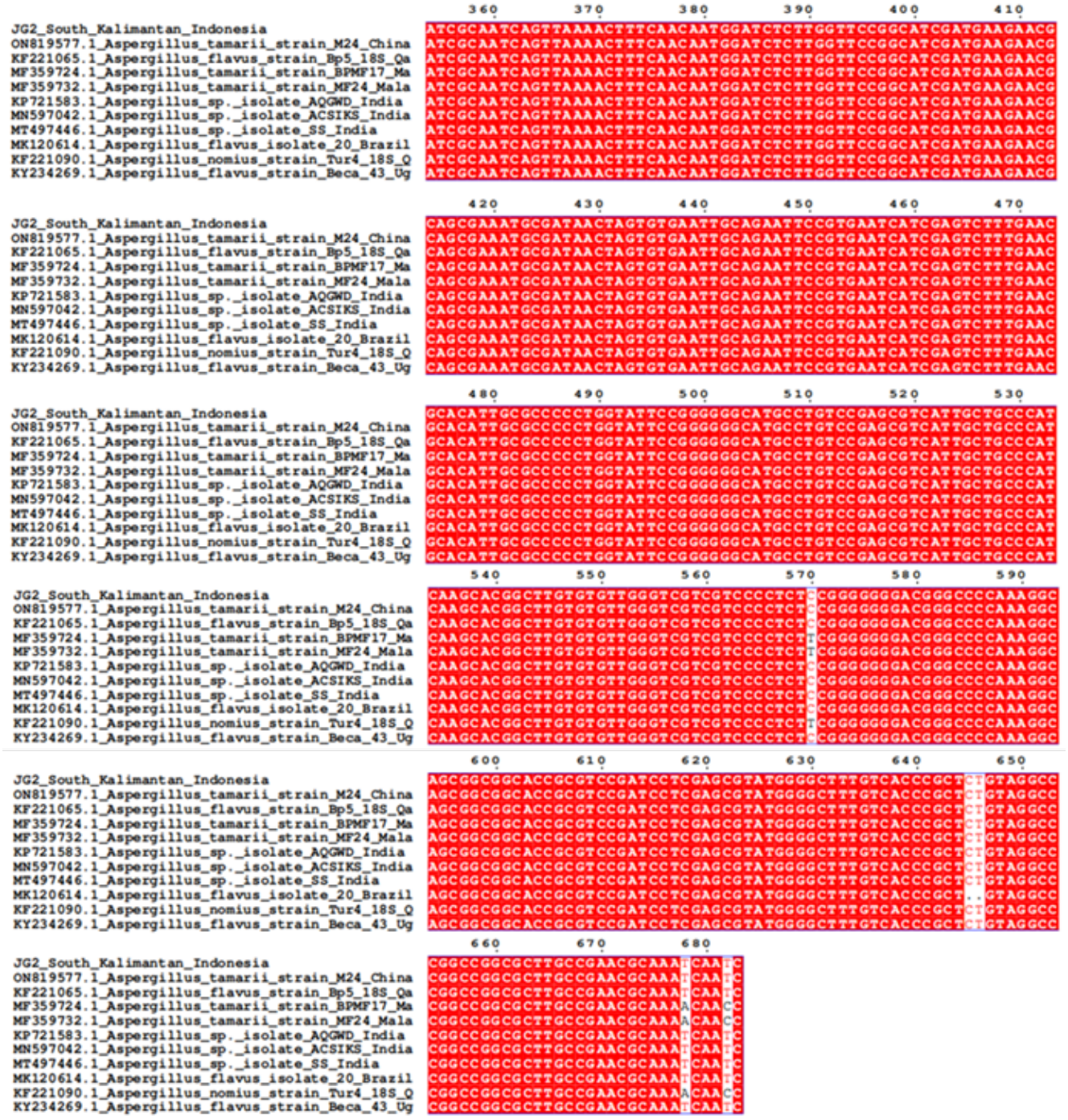


Figure 6. Multiple sequence alignment (MSA) between JG2 samples from Satui District, South Kalimantan, and DNA sequences from the NCBI database.

field and during storage. In the field, only 1 out of 20 inoculated plants developed cob rot symptoms, while none of the control plants showed signs of infection (Figure 8). After harvest, however, symptoms appeared in storage: inoculated cobs developed infection within 13 days, and 16 of 20 previously healthy cobs later showed *A. flavus* symptoms by day 20 (Figure 9). This secondary contamination was likely due to storing healthy and inoculated cobs together.

Field fungi may persist on grains at harvest but usually decline during storage, while post-harvest fungi such as *A. flavus* become dominant under high-moisture conditions, especially at relative humidity above 90%. In storage, infections caused by *F. verticillioides* are second only to those caused by *A. flavus* (Pakki, 2016).

Effective prevention of *A. flavus* contamination must begin in the field. Recommended practices include controlling foliar diseases, ensuring adequate nutrient supply—particularly calcium—and reducing drought stress during the critical reproductive period (3–6 weeks

before harvest). Resistant corn varieties can also reduce susceptibility.

Post-harvest strategies focus on limiting fungal growth by harvesting under favorable weather, promptly removing damaged or immature cobs, and drying kernels to below 9% moisture before storage. Grains should then be stored in airtight containers in clean, well-ventilated facilities (Kasno, 2009).

Aflatoxin Contamination in Corn Feed. ELISA analysis confirmed that JG2 produced aflatoxins in corn kernels. The detected concentration exceeded 8.0 ppb, surpassing the safety thresholds of Singapore and Malaysia (2.0 ppb) but remaining below the Indonesian National Standard (50 ppb for feed; 20 ppb for food) (Table 4).

Similar findings have been reported in other

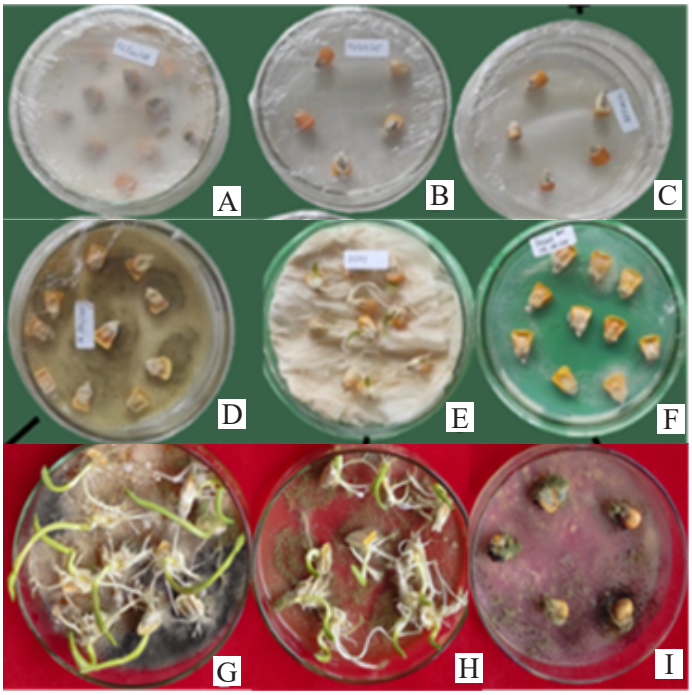


Figure 7. Pathogenicity test on corn seeds. A. Diseased seed cultured on filter paper, 2-day incubation; B–C. Diseased seed cultured on PDA medium, 2-day incubation; D. Healthy seed inoculated with *A. flavus* isolate and cultured on PDA medium, 2-day incubation; E. Healthy seed cultured on filter paper, 2-day incubation; F. Healthy seed cultured on PDA medium, 2-day incubation; G. Healthy seed cultured on filter paper, 10-day incubation; H. Healthy seed inoculated with *A. flavus* isolate and cultured on PDA medium, 10-day incubation; I. Diseased seed cultured on PDA medium, 10-day incubation.

Figure 8. Pathogenicity test of *A. flavus* on corn plants.

Figure 9. Symptoms of *A. flavus* infection on inoculated plants after 13 days in storage.

Table 4. Aflatoxins concentrations in feed corn samples from Satui, Kotabaru Regency, South Kalimantan

No	Sample	Absorbance	Concentration (ppb)
1	Standar 0 ppb	2.29	Nd
2	Standar 4 ppb	2.02	4.17
3	Standar 10 ppb	1.56	8.76
4	Standar 20 ppb	0.70	22.65
5	Standar 40 ppb	0.35	38.65
6	Sampel 1	2.25	7.99

countries. For example, Mongkon et al. (2017) observed higher aflatoxin B1 (AFB1) contamination in corn compared to processed feed, while Zaboli et al. (2010) reported contamination increases during long-term storage. Chronic consumption of contaminated feed has been linked to reduced egg production and toxin residues in animal products (Salwa et al., 2009; Herzallah, 2013).

***Aspergillus* Infections in Stored Corn and Implications for Plant Protection.** *Aspergillus flavus* is a major storage fungus causing cob rot and aflatoxin contamination (Pakki, 2016). Favorable environmental conditions—high temperature, high kernel moisture, and relative humidity above 90%—promote fungal proliferation and toxin production (Pitt & Miscamble, 1995; Robertson, 2005). Aflatoxin contamination may begin within 24 hours after infestation (Gwinner et al., 1996).

Post-harvest handling is therefore critical. Delayed drying or leaving harvested corn in the field substantially increases aflatoxin accumulation (Hell et al., 2003). High levels of aflatoxin have caused severe aflatoxicosis outbreaks, including human fatalities in Kenya (Lewis et al., 2005, Probst et al., 2007) and Tanzania (Kamala et al., 2018).

Efforts to reduce aflatoxin in maize have focused on improved cultivation, post-harvest handling, grain drying, and biological control (Hell et al., 2000; 2003; Bandyopadhyay et al., 2016). Aflatoxin levels vary with planting season, maize variety, and storage conditions, particularly humidity and kernel moisture, which strongly influence pathogen infection and disease spread (Williams et al., 2020).

In Indonesia, monitoring of corn samples has revealed alarming contamination levels. A 2016 survey in West Java found aflatoxin concentrations ranging from 25.5–355 µg/kg in farmer samples and 25.5–655 ppb in trader samples, both exceeding the SNI safety limits (BPMPT, 2016).

CONCLUSION

Aspergillus flavus infects corn cobs in the field and can subsequently contaminate healthy corn kernels.

The fungus is also capable of contaminating kernels during storage when conditions are favorable. Further testing confirmed that *A. flavus* produced aflatoxins exceeding 8.00 ppb, which is above the standard safety threshold of 2.00 ppb. Therefore, further studies and proper management strategies for stored corn kernels are essential to reduce aflatoxin levels and ensure the safety of uncontaminated kernels in storage warehouses.

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AUTHORS' CONTRIBUTIONS

SS and YM designed and supervised the research. SA performed the experimental work and molecular analysis. MM conducted isolation and sample preparation for fungal molecular identification. MIP, MS, and LA collected corn seed samples from the field. SS and YM prepared the report and drafted the manuscript. All authors contributed to the research workflow, data analysis, and interpretation, as well as manuscript revision. All authors have read and approved the final version of the manuscript.

COMPETING INTEREST

The authors declare that they have no competing interests, financial or non-financial, related to the conduct of this study or the preparation of this manuscript.

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